



# CORRELATION STUDY OF RETINOL BINDING PROTEIN(-4), NESFATIN AND THYROID HORMONES IN COLORECTAL CANCER IRAQI MALE PATIENTS

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## Abstract

The third most ordinarily cancer type diagnosed in male and is Colorectal cancer (CRC) and it is widely spread in developed countries. Most of CRC arises from development of adenomatous polyps.

The current study aimed to determine whether serum retinol binding protein 4 (RBP4) and Nesfatin-1 can be used as a novel biomarker for diagnosis of CRC. Nesfatin-1, RBP4 and Thyroid Hormones (T3, T4 and TSH) levels were measured in fifty sera of male patients suffering from CRC before chemotherapy initiation treatment as G1, G2 after first chemotherapy cycle dose and G3 after second chemotherapy cycle dose compared with twenty five male volunteers as a control G4.

The results showed a significant increased in RBP 4 concentration in G3 and a significant elevated in nesfatin-1 in G2 than other groups. The correlation between RBP4 and TSH was a high significant negative (-ve) relation while with nesfatin-1 gave a significant positive (+ve) correlation in G1. The results also found significant positive (+ve) correlation between RBP-4 with, nesfatin-1 in G2 and there was significant negative (-ve) correlation between RBP4 with nesfatin-1 in G3

**Key words:** Colorectal cancer (CRC), Nesfatin-1, RBP4 and Thyroid Hormones.

## Introduction

Retinol binding protein4 (RBP4) is an adipokine which produced from adipocytes, it is basically transporting retinol from the liver to others tissues(Patrick H. Dessein *et al.*, 2014) and inducing gluconeogenesis via stimulating phosphoenolpyruvate carboxykinase. Increasing levels of RBP4 was observed in several complications such insulin resistance (IR) which correlated with obesity and type 2 diabetes (T2D) (Yang Q *et al.*, 2005). Also this increasing complicated with metabolic syndrome(Graham TE *et al.*, 2006; Lim S *et al.*, 2010) and cardiovascular diseases(Ingelsson E *et al.*, 2013; Won J.C. *et al.*, 2012).

Nesfatin-1 is a neuro polypeptide secreted from hypothalamus (Shinsuke OI *et al.*, 2006), discovered in 2006 by the Japanese scholar OH-I. Nesfatin-1 is known as appetite suppressor, found highly expressed in the peripheral adipose tissues and central nervous system, pancreas and gastric mucosa. It plays an important role in food intake, carbohydrates metabolism, energy

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regulator, depression, anxiety and also in the cancer's pathogenesis (Wang GH *et al.*, 2016); (Goebel-Stengel M. *et al.*, 2011). Nucleobindin-2 (NUCB-2) is the precursor of Nesfatin-1 and nesfatin-1/NUCB-2 is complicated with regulating tumorigenesis, tumor development and metastasis (Xu H *et al.*, 2018; Ziwei Wei *et al.*, 2019), moreover increases cell migration and invasion in CA colon (Jung-Yu Kan *et al.*, 2016).

Thyroid hormones consist the main hormone; the inactive form Tetraiodothyronine or thyroxine (T4) and active form triiodothyronine (T3) (Olga Rostkowska *et al.*, 2019). These hormones are synthesized and secreted in response to thyroid stimulating hormone (TSH) which secreted by pituitary gland (Eilon Krashin *et al.*, 2019). Thyroid hormones play an important role in cellular processes regulations, cell proliferation, differentiation, metabolism and apoptosis (Boursi, B. *et al.*, 2015). Thyroids dysfunction (hyper or hypothyroidism) data related to CRC provided contradictory results, some of these reveled that patients with thyroid dysfunction had CRC risk and both hyperthyroidism and untreated hypothyroidism were correlated with increased risk of

CRC incidence (Boursi, B. *et al.*, 2015). Other revealed that elevation TSH was association with CRC(Chan, Y.X. *et al.*, 2017). It was found that thyroid hormones complex interactions and their associated receptors with normal and neoplastic CRC tissues(Abby L'Heureux *et al.*, 2019).

The third most ordinarily cancer type diagnosed in the world wide is Colorectal cancer (CRC) (Siegel, R.L. *et al.*, 2015). Most of CRC arises from development of adenomatous polyps originating from the intestinal lining (Brenner, H. *et al.*, 2007). CRC is affected by several factors but the environmental and genetic seems the predominated increased risk of CRC influence than others (Prashanth Rawla *et al.*, 2019). Most CRC patients were older age, over 50 years and more popular in the male (Nakagawa, H. *et al.*, 2016; Caldarella, A. *et al.*,

2013), although recent data revealed that increasing CRC incidence under 50 years old and decreasing in older people(Ward, E.M. *et al.* 2019; Meester, R.G.S. *et al.*, 2019) *via* the later stage diagnosed.

This study was conducted to estimate RBP4, Nesfatin-1 and thyroid hormones (T3, T4, with TSH) levels in Iraqi male patient's sera with colorectal cancer and to find the correlation between these variants.

### Materials and Methods

#### Patient study

The serum of (50) males patients suffering from colorectal cancer with age range between (50-65) years old were collected in this study from Oncology Teaching Hospital in Medical City in Baghdad for duration time January, 2019 to December, 2019. The patient's sera were divided into three groups as following:

G1: fifty male patients with CRC before initiation

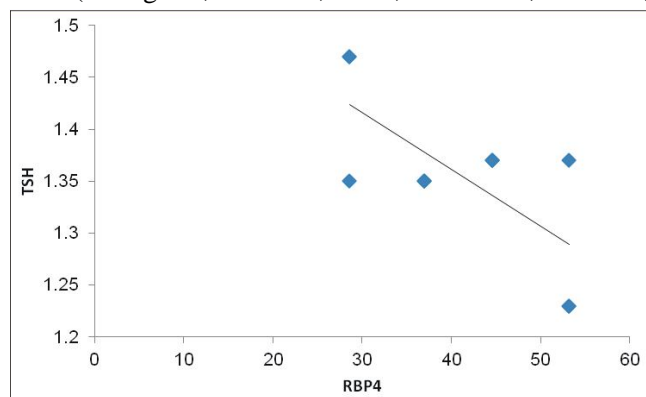


Fig. 1: Correlation between RBP4 and TSH in male G1.

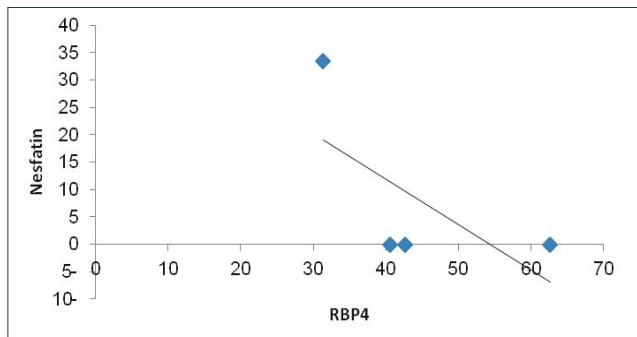


Fig. 4: Correlation between RBP4 and Nesfatin in male G3.

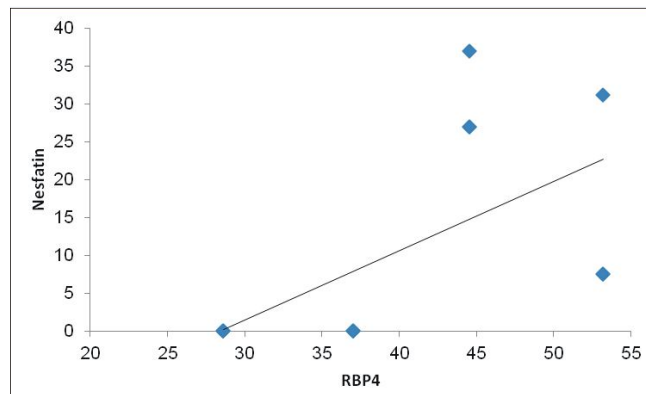


Fig. 2: Correlation between RBP4 and Nesfatin in male G1.

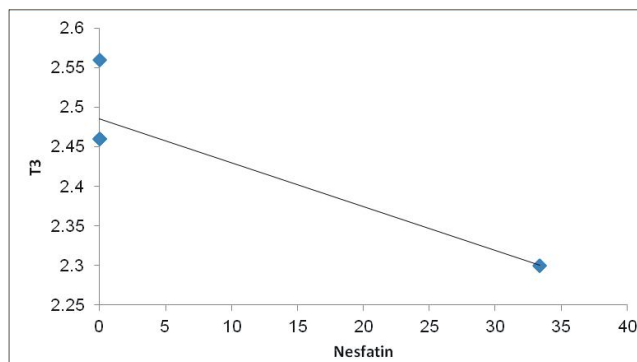


Fig. 5: Correlation between Nesfatin and T3 in male G3.

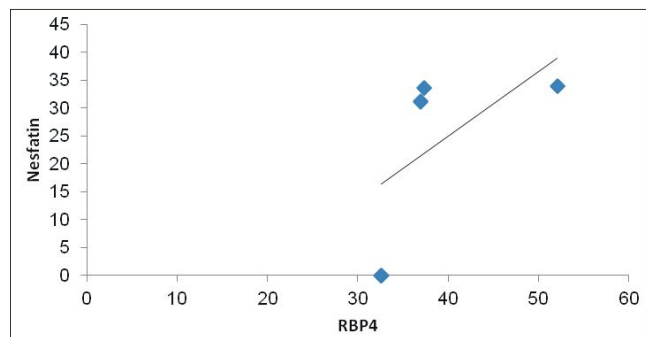


Fig. 3: Correlation between RBP4 and Nesfatin in male G2.

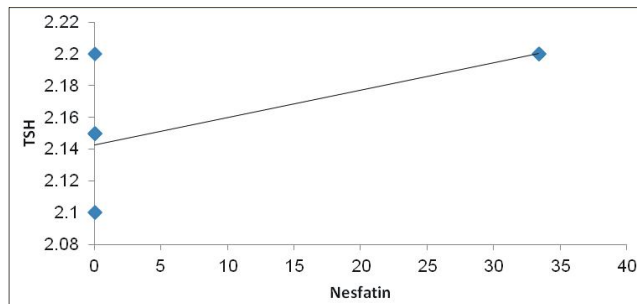


Fig. 6: Correlation between Nesfatin and TSH in male G3.

chemotherapy.

G2: G1 patients after three weeks of first cycle chemotherapy.

G3: G1 patients after three weeks of second cycle chemotherapy.

The healthy control consist of (25 male) with the same age of patients considered as (G4).

Nesfatin-1, RBP4 estimated by ELISA kit No. E-EL-H2373,E-EL-H1581 respectively. Thyroid hormones (T3, T4 and TSH) were determined by Electrochemiluminescence (ECL) on Roche cobas e 411.

**Statistical Analysis**

For statistical analyses, SPSS version (prism® 7 and Microsoft excel 2013) (Elliott, A.C. *et al.*, 2007) was used and standard deviation (mean±SD) test. The measured values of  $P \leq 0.05$  were considered as significant. Correlation analysis was performed by Person correlation test and r was calculated for the variants.

**Results and Discussion**

The obtained data in the table 1, showed a significant elevated in RBP4 (44.3±11.9pg/ml) in G3 as compared with G1, G2 and G4 whereas there was non significant lowering RBP4 in G1 and G2 as compared with G4.

RBP4 is the transfer for vitamin A (retinol) from the liver, main storage site and other tissues *i.e.* adipose tissue and lung to other parts of the body. When RBP bound with ROH formed a holo-RBP, this complex with its receptor STRA6 considered as potent oncogenes and has been reported that STRA6 is upregulated in several human cancers, including ovarian, colorectal and endometrium cancers (Daniel, C. *et al.*, 2014). The increasing level of RBP4 in the patient after second dose of chemotherapy G3, may be due to protect the colon from cancer cells. Also table 1, showed significant

increased in levels of nesfatin-1 in G2 (24.66±1.49 pg/ml) than other groups and showed a significant lowering concentration in G4 (0.01±0.004 pg/ml) as compared with other groups. The elevated levels of nesfatin-1 after first dose of chemotherapy due to the function of nesfatin-1 that similar to the tumor developments and have not been studied in colon cancer, in previous study showed non significant difference between healthy and CRC patients (Lambadiari, V. *et al.*, 2014). The increasing possibility of nesfatin-1 may be due to inhibit cell proliferation through reducing mTOR phosphorylation and activating protein kinase as showed in previous study (Chih-Chien Chou *et al.*,2014).

The high level of nesfatin-1 after taking chemotherapy belong to the treatment enhanced increasing of nesfatin to stop cell cancer from proliferation of that cancer. It was evidenced that nesfatin-1/NUCB-2 increases cell migration, invasion and epithelial-mesenchymal transition in CA colon. NUCB-2 expression correlated with early metastasis and this expression was higher rate in tumor tissues from in nontumor tissues (Lambadiari, V. *et al.*, 2014).

Also the data in table 1, showed the levels of thyroid hormones (T3, T4 and TSH) in Iraqi patients with colorectal cancer. Thyroid hormones have been shown to affect several pathways in cancer development which including CRC (Siegel, R.L. *et al.*, 2015). The decreasing levels of thyroid hormones in patients of G1 without taking chemotherapy treatment as a compared with other groups. The appearance of hypothyroidism in colorectal cancer Iraqi patients, may be due to the age older than 50 years (Siegel, R.L. *et al.*, 2015). In addition regulation of body metabolism thyroid hormones played an important pathways in cell proliferation and differentiation (Kress, E. *et al.*, 2010). The alteration in thyroid hormones and their receptor have been associated with CRC (Hörkkö,

**Table 1:** Concentrations of RBP4, Nesfatin-1 and thyroid hormones (T3, T4 and TSH) in sera of male patients and control groups.

P value						Control group (No. 25) G4	Patients group (No. 50)				Parameters
G3 vs. G4	G2 vs. G4	G2 vs. G3	G1 vs. G4	G1 vs. G3	G1 vs. G2		G3	G2	G1		
0.039S	0.25N s	0.04S	0.4Ns	0.07S	0.7N s	41±7.2	44.3± 11.9	39± 7.68	40.84± 9.50	RBP4 (pg/ml)	
0.8Ns	0.000h s	0.007S	0.02S	0.6N s	0.0.3s	0.01±0.004	8.35± 1.5	24.66± 1.49	11.4± 0.14	Nesfatin-1 ( pg/ml )	
0.000h s	0.000h s	0.000h s	0.000h s	0.000h s	0.000h s	2.0±0.004	2.44± 0.09	3.24± 0.17	1.89± 0.07	T3 (nmol/L)	
0.000h s	0.000h s	0.000h s	0.000h s	0.000h s	0.000h s	140±0.03	166.35± 0.88	152.4± 0.91	130.4± 0.98	T4 (nmol/L)	
0.000h s	0.000h s	0.000h s	0.000h s	0.000Hs	0.000h s	2.0±0.06	2.16± 0.039	1.18± 0.04	1.36± 0.08	TSH (nmol/L)	

**Table 2:** Correlations between RBP4 and some bio-chemical parameters in sera of CRC patients male before taking the dose of chemotherapy.

Nesfatin	TSH	T4	T3	Parameters	
0.597	-0.894	0.326	-0.186	R	RBP4
0.041S	0.000 h s	0.358Ns	0.585Ns	P	
	-0.321	-0.021	0.120	R	Nesfatin
	0.367Ns	0.954Ns	0.725 Ns	P	

S = Significant, Ns = Non significant, s = high significant

T.T. *et al.*, 2006) and it may played a tumor suppressor-type role in malignancy progression. T4 has been shown to promote  $\beta$ -catenin activation and cell proliferation in CRC (Lee, Y.S. *et al.*, 2018) and thyroid hormone binding off cell surface receptor has been show to lead increased tumor cell proliferation and angiogenesis (Chan, Y.X. *et al.*, 2018). In addition T3 play important role to activated TR $\alpha$ 1 which is directly modulated the transcription of the  $\beta$ -catenin gene which is associated with colon cancer development (Lin, H.Y. *et al.*, 2016). So thyroid hormones have been shown to promote RCR stem cell depletion in CRC without treatment with chemotherapy (Cicatiello, A.G. *et al.*, 2017). But after chemotherapy treatment it be showed the affecting in metabolism of thyroid hormones by increasing T3, T4 and decreasing TSH.

Tables 2, 3 and 4, referred to the data correlation between nesfatin-1 and RBP4 with (T3, T4 and TSH) for Iraqi male patients with CRC in G1, G2 and G3 respectively. There was a high significant negative (-ve) correlation between RBP4 with TSH, significant positive (+ve) correlation with nesfatin-1 and non significant negative correlation with T3 and positive (+ve) correlation with T4 while there were non significant negative correlation (-ve) between nesfatin-1 and (TSH and T4) and non significant positive correlation with T3 in G1 patients before chemotherapy dose.

Table 3, showed significant positive correlation (+ve) between RBP4 and nesfatin-1 and non significant positive correlation (+ve) with (TSH, T3 and T4) and between nesfatin-1 with T4. There were non significant negative (-ve) correlation between TSH and T3 in G3 patients

**Table 3:** Correlations between RBP4 and some bio-chemical parameters in sera of CRC patients male after taking first dose of chemotherapy.

Nesfatin	TSH	T4	T3	Parameters	
0.595	0.016	0.036	0.091	R	RBP4
0.041S	0.373 Ns	0.922Ns	0.791Ns	P	
	-0.253	0.225	-0.491	R	Nesfatin
	0.481Ns	0.531Ns	0.125 Ns	P	

S = Significant, Ns = Non significant, s = high significant

**Table 4:** Correlations between RBP-4 and some bio-chemical parameters in sera of CRC patients male after taking second dose of chemotherapy.

Nesfatin	TSH	T4	T3	Parameters	
-0.655	-0.152	-0.015	0.500	R	RBP4
0.021S	0.674 Ns	0.967Ns	0.117Ns	P	
	0.700	-0.039	-0.913	R	Nesfatin
	0.024S	0.915Ns	0.000 h s	P	

S = Significant, Ns = Non significant, s = high significant

after first dose treatment of chemotherapy.

Table 4, showed significant negative (-ve) between RBP4 with nesfatin-1 and non significant positive (+ve) negative correlation (-ve) with T3 and T4 respectively. Also there were non-significant negative (-ve) correlation between nesfatin- 1 with T4 and high significant negative (-ve) with T3 and significant positive correlation (+ve) with TSH in G3 patients after second dose treatment of chemotherapy.

### Conclusion

The results from present research appears a higher circulating levels of RBP4 after second dose of chemotherapy and higher amount in nesfatin-1 after first dose of chemotherapy which may be considered an indicator of maintenance of colon carcinogenesis and a good biomarker for indicator of CRC.

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